(12) UK Patent Application (19) GB (11) 2 229 814 (13) A

(43) Date of A publication 03.10.1990

(21) Application No 9003909.0

(22) Date of filing 21.02.1990

(30) Priority data
(31) 8904129 (32) 23.02.1989 (33) GB

(51) INT CL[5]
G01N 33/50

(52) UK CL (Edition K)
G1B BBN BCD

(56) Documents cited
None

(58) Field of search
UK CL (Edition K) G1B BBE BBN BCD BCX
INT CL[5] G01N
Online databases: WPI, CAS-ONLINE

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(54) Detection or investigation of DNA

(57) An apparatus for detecting or investigating DNA comprises a thermally insulated cabinet 10 defining a chamber which is heated by an electrical resistance heating element, the heating effect of which is distributed throughout the chamber by means of a heat spreading plate 14, the temperature within the cabinet being maintained at about 65°C by a thermostat 16. The cabinet 10 has an electric motor 18, the drive shaft of which carries a pinion 20 driving three spur gears arranged horizontally in line, the pinion 20 driving the central spur gear which meshes with and drives the two outer spur gears. Each spur gear is mounted on (and drives) a corresponding one of three shafts the ends of which project into the heated cabinet 10 and form stub shafts 24.

Each stub shaft 24 engages the outer periphery of an end plug 26 which closes a corresponding one of three glass tubular receptacles 28 which are sealed at their opposite ends by similar plugs 30.

In use, as each tube 28 is rotated the DNA, smeared on a carrier, is brought into intimate contact with a radioactive probe solution introduced into the receptacle and which has an affinity with the sequence being detected or investigated.

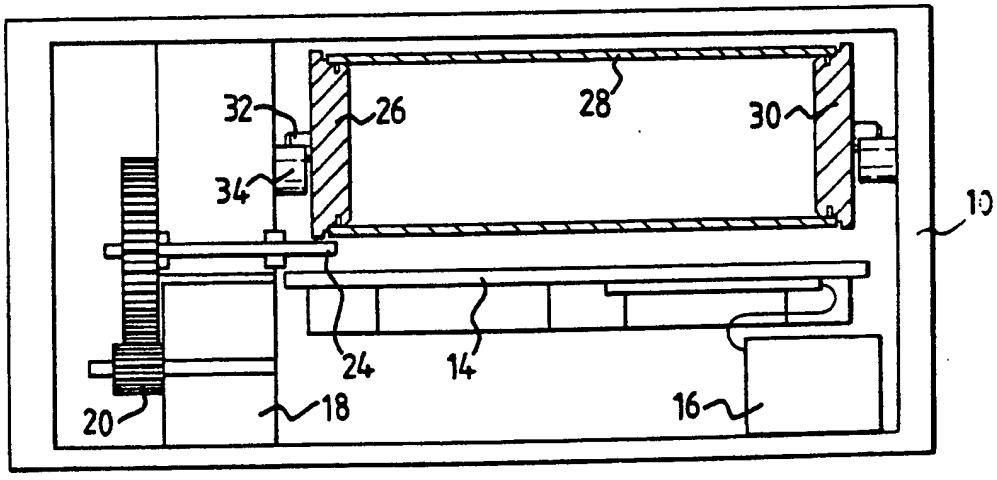

FIG.2

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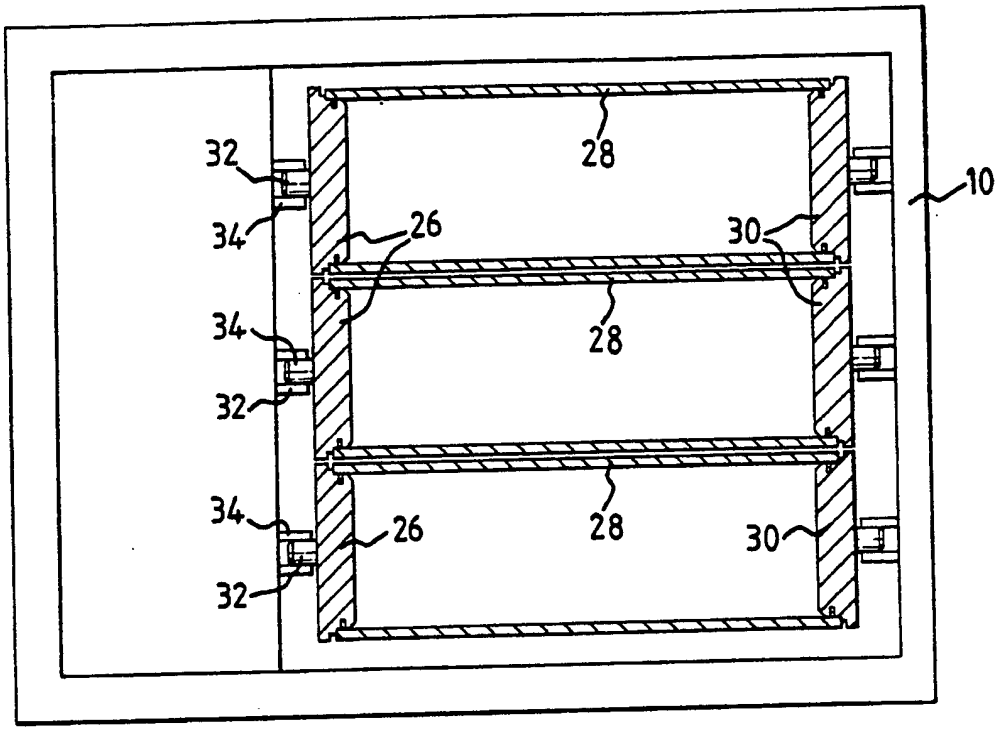
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FIG.1

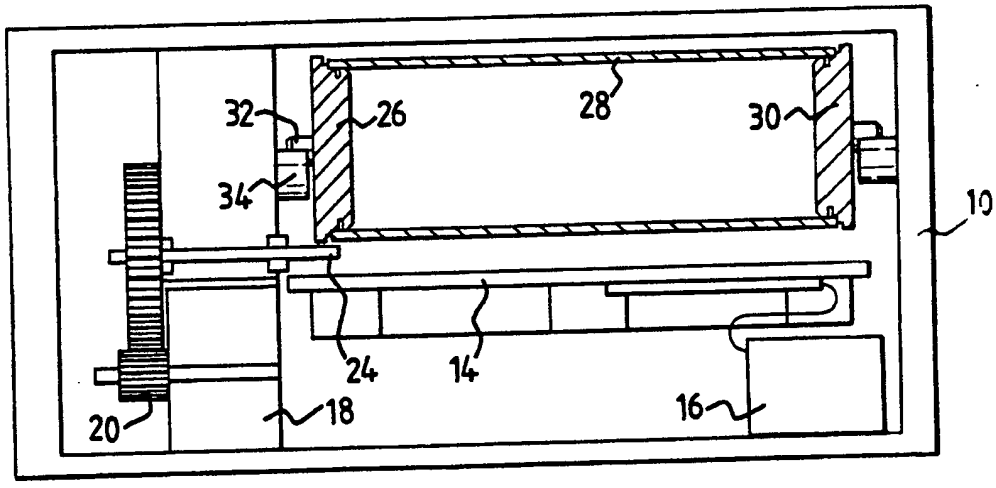
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FIG.2

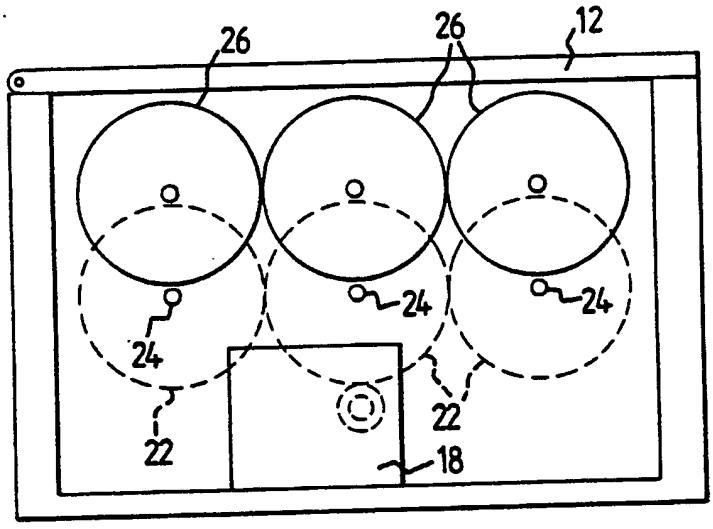

FIG.3

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Title: Detection or Investigation of Proteins

Field of invention

This invention relates to the detection or investigation of proteins, for example in research into the genetic code and in the identification of proteins which bind to or have affinity with other proteins.

Background to the invention

An important task that forms part of most genetics research is genetic mapping, the term given to trying to break the DNA Code which is the basis of the hereditary information of nucleic acids. This area of research holds the key to important medical advances, such as the discovery of treatments for leukemia and cancer. A step within a generally used procedure carried out by researchers in this field is called hybridisation. In this method a sheet of nylon or other carrier is smeared with the DNA molecules to be detected or investigated. The carrier bearing the DNA molecules is placed with a small quantity of radioactive probe solution in a plastic bag from which the air is expelled. The bag is sealed and after an appropriate time interval the carrier is removed and used to expose a radiographic plate which shows the regions of high concentration of probe and therefore gives information to the researcher about the proteins present

in the DNA under investigation. The invention aims to provide an improved method and apparatus for bringing the protein under investigation into contact with the radioactive probe solution.

Summary of the invention

According to one aspect the invention provides apparatus for detecting or investigating proteins, comprising a chamber, means for maintaining the chamber within a predetermined temperature range, an elongate openable and closable receptacle for holding a carrier for a protein, means for supporting the receptacle within the chamber with the longitudinal axis of the receptacle substantially horizontal, and means for rotating the receptacle about its longitudinal axis, whereby in use as the receptacle rotates the protein is brought into intimate contact with a radioactive probe solution which is introduced into the receptacle and which has an affinity with the protein being detected or investigated.

The receptacle may be tubular and may be openable and closable at at least one end.

The use of a tubular receptacle, with means for supporting the receptacle and rotating the receptacle within the temperature controlled chamber, results in the protein under detection or investigation being brought into more intimate and uniform contact with the probe solution.

The chamber is preferably defined by a cabinet which has an access door or lid and which is conveniently heated by an electrical resistance heating element under thermostatic control, typically being maintained at a temperature in the range 62°C to 68°C, preferably about 65°C.

The receptacle preferably has a removable end plug which

makes a liquid tight seal at said one end of the tubular receptacle which is preferably of glass. The receptacle is preferably a cylindrical tube closable at both ends by respective end plugs, facilitating insertion and removal of the carrier. At least one end plug may have a central hole, so that fluid can be injected into the receptacle without the need to remove the plug.

The means for supporting the tubular receptacle preferably comprise bearing supports which cradle cylindrical spigots projecting outwardly from the end plugs, the bearing supports being upwardly open to enable the closed receptacle to be laid into or removed from the bearing supports.

The means for rotating the receptacle preferably engage the circular rim of one of the end plugs so as frictionally to drive the latter, and in a preferred embodiment a stub shaft is rotatably driven by an electric motor and projects into the chamber where the stub shaft frictionally engages the outer periphery of the end plug.

The chamber may accommodate more than one tubular receptacle, in which case the receptacles are preferably of the same size and supported in parallel side by side relationship, each being driven by a corresponding one of a plurality of stub shafts. The stub shafts preferably drive the receptacles so that any two adjacent receptacles are driven in mutually opposite directions, and in a preferred embodiment the end plugs of adjacent tubular receptacles engage so that each tubular receptacle may be regarded as being driven not only by its corresponding stub shaft but also by adjacent receptacle(s), thereby minimising slippage and maintaining even rotation. The

rotational speed is typically of the order of four revolutions per minute.

According to another aspect of the invention there is provided a method of detecting or investigating proteins, comprising applying the protein to be detected or investigated to a carrier, inserting the carrier with the protein thereon into a receptacle, introducing a radioactive probe solution into the receptacle and sealing the latter, rotating the receptacle substantially about its longitudinal axis within a chamber the temperature of which is thermostatically controlled, withdrawing the carrier from the receptacle and exposing a radiographic plate from the carrier thereby deriving information about the protein to be detected or investigated.

The protein may be applied to the carrier by smearing.

The method according to the invention preferably includes a pre-hybridisation step which involves introducing the carrier and protein into the receptacle with a pre-hybridisation solution which has the effect of improving the contrast and providing a clearer image on the eventual radiograph. After pre-hybridisation for an initial period of time (during which the tubular receptacle accommodating the protein and pre-hybridisation solution is rotated in the chamber under the controlled temperature conditions), the radioactive probe solution is added to the tubular receptacle which is then resealed and replaced in the chamber where it is rotated for a further period of time.

The invention will now be described by way of example with reference to the accompanying drawings, in which:
Figure 1 is a plan view of apparatus according to the invention with a lid removed,
Figure 2 is a diagrammatic side view of the apparatus of

Figure 1, and
Figure 3 is a diagrammatic end view of the apparatus of Figure 1.

The apparatus comprises a thermally insulated cabinet 10 having a hinged lid 12 (Figure 3) which is omitted from Figure 1 to show internal detail. The chamber within the cabinet 10 is heated by an electrical resistance heating element, the heating effect of which is distributed throughout the chamber by means of a heat spreading plate 14, best shown in Figure 2. The temperature within the cabinet is maintained at about 65°C by means of a thermostat 16.

The cabinet 10 also incorporates an electric motor 18, the drive shaft of which carries a pinion 20 driving three spur gears 22 are arranged horizontally in line, and the pinion 20 drives the central spur gear 22 which meshes with and drives the two outer spur gears 22. Each spur gear 22 is mounted on (and drives) a corresponding one of three shafts the ends of which project into the heated cabinet 10 and form stub shafts 24.

Each stub shaft 24 engages the outer periphery of an end plug 26 which closes a corresponding one of three glass tubes 28 which are sealed at their opposite ends by similar plugs 30. Each plug 26 or 30 has an O-ring seal and is formed with a projecting spigot 32 which serves to support the corresponding tube 28 in bearing supports 34 projecting into the cabinet from opposite ends thereof. One of the plugs sealing each tube has a central hole (through the spigot 32), so that fluid can be injected into the tube without the need to remove the plug. Each support 34 presents an upwardly open semi-circular bearing

surface within which the corresponding spigot 32 is cradled for rotation of the spigot 32, enabling the tubes 28, with their end plugs 26, 30, to be put into or lifted out of the cabinet 10. Hence, the three tubes 28 are supported in parallel side by side relationship, with the longitudinal axis of each tube 28 being horizontal. Adjacent end plugs 26, 30 engage so that when the motor 18 is energised the stub shafts 24 rotate the tubes about their respective longitudinal axes, drive also being applied from one tube to the adjacent tube by means of the engaging end plugs 26 and 30. It will be appreciated that any adjacent pair of tubes 28 rotate in mutually opposite directions.

The described apparatus is used to investigate protein in DNA in the following manner. The DNA is smeared on a nylon paper carrier, and the carrier and DNA are inserted into one of the tubes after removal of one (or both) of the end plugs therefrom. A pre-hybridisation fluid is added to the tube 28 and the latter is then resealed by re-attachment of the end plug. The tube 28, together with the carrier bearing the DNA and the pre-hybridisation fluid, is then placed in the cabinet 10 and the lid is closed. The heating element and the electric drive motor are energised so as to cause the receptacle to be rotated at about 4 rpm in a controlled temperature of about 65°C. This initial pre-hybridisation stage takes one to two hours and it has the effect of reducing background "noise" in the resulting radiograph, thereby improving contrast and providing a clearer image.

After pre-hybridisation the tube is removed from the cabinet, a small amount of radioactive probe solution is added to the tube by injection of the solution through the

hole in one of the end plugs. The tube is then replaced in the cabinet. The tube is then rotated for a further period of about five hours at the controlled temperature of 65°C. The carriers are then rinsed, taken out of the tubes and then used to expose a radiographic plate which shows the regions of high concentration of probe. The probe has an affinity for certain proteins under investigation, so the radiographic plate reveals the presence of protein to be investigated.

Because the carrier bearing the DNA is supported in a tube which is rotated evenly about its horizontal axis, less pre-hybridisation fluid and less probe solution are necessary than with the prior methods using plastic bags. The liquid in the tube forms an even ribbon and steady rotation of the tube results in an even and thorough exposure of the DNA, first to the pre-hybridisation liquid and then to the probe solution.

The described method provides the opportunity to quicken the process by increasing the probe concentration. Other advantages of the described method over existing methods are:

-it is quicker to load and unload each tube
-it protects the user from potentially harmful beta radiation.
-the costs of the reagents will be substantially less.
-less capital outlay will be needed to carry out hybridisations than at present.
-the time taken for the process can be shortened from the conventional 18 hours to about 6 hours.

CLAIMS

1. Apparatus for detecting or investigating proteins, comprising a chamber, means for maintaining the chamber within a predetermined temperature range, an elongate openable and closable receptacle for holding a carrier for a protein, means for supporting the receptacle within the chamber with the longitudinal axis of the receptacle substantially horizontal, and means for rotating the receptacle about its longitudinal axis, whereby in use as the receptacle rotates the protein is brought into intimate contact with a radioactive probe solution which is introduced into the receptacle and which has an affinity with the protein being detected or investigated.

2. Apparatus according to Claim 1, the receptacle being tubular and openable and closable at at least one end.

3. Apparatus according to Claim 1 or Claim 2, the chamber being defined by a cabinet which has an access door or lid.

4. Apparatus according to any preceding Claim, the means for maintaining the temperature of the chamber within a predetermined temperature range comprising an electrical resistance heating element under thermostatic control and adapted to maintain a temperature in the range 62°C to 68°C.

5. Apparatus according to Claim 4, the heating element being adapted to maintain a temperature of 65°C in the chamber.

6. Apparatus according to any of Claims 2 to 5, the receptacle having a removable end plug which makes a liquid-tight seal at said one end of the tubular receptacle.

7. Apparatus according to any preceding Claim, the receptacle being made of glass.

8. Apparatus according to any of Claims 2 to 7, the receptacle comprising a cylindrical tube closable at both ends by respective end plugs, facilitating insertion and removal of the carrier.

9. Apparatus according to Claim 8, at least one end plug having a central hole, whereby fluid can be injected into the receptacle without the need to remove the plug.

10. Apparatus according to Claim 8 or Claim 9, the means for supporting the receptacle comprising bearing supports which cradle cylindrical spigots projecting outwardly from the end plugs, the bearing supports being upwardly open whereby to enable the closed receptacle to be laid into or removed from the bearing supports.

11. Apparatus according to any of Claims 8 to 10, the means for rotating the receptacle engaging a circular rim of one of the end plugs so as frictionally to drive the latter.

12. Apparatus according to Claim 11, comprising a stub shaft rotatably driven by an electric motor and projecting into the chamber so that the stub shaft frictionally engages the outer periphery of the end plug.

13. Apparatus according to Claim 12 or Claim 13, the chamber being adapted to accommodate more than one tubular receptacle.

14. Apparatus according to Claim 13, the receptacles being of substantially the same size and supported in parallel side by side relationship, each being driven by a corresponding one of a plurality of stub shafts.

15. Apparatus according to Claim 14, the stub shafts being adapted to drive the receptacles so that any two adjacent receptacles are driven in mutually opposite directions.

16. Apparatus according to Claim 15, the end plugs of adjacent tubular receptacles being in engagement so that each tubular receptacle may be regarded as being driven not only by its corresponding stub shaft but also by adjacent receptacle(s), thereby minimising slippage and maintaining even rotation.

17. Apparatus according to Claim 16, the motor being adapted to provide a rotational speed being typically of the order of four revolutions per minute.

18. Apparatus for detecting or investigating proteins, substantially as hereinbefore described with reference to the accompanying drawings.

19. A method of detecting or investigating proteins, comprising applying the protein to be detected or investigated to a carrier, inserting the carrier with the protein thereon into a receptacle, introducing a radioactive probe solution into the receptacle and sealing the latter, rotating the receptacle substantially about its longitudinal axis within a chamber the temperature of which is thermostatically controlled, withdrawing the carrier from the receptacle and exposing a radiographic plate from the carrier thereby deriving information about the protein to be detected or investigated.

20. A method according to Claim 19, the protein being applied to the carrier by smearing.

21. A method according to Claim 19 or Claim 20, including a pre-hybridisation step comprising introducing the carrier and protein into the receptacle with a pre-hybridisation solution which has the effect of improving the contract and providing a clearer image on the eventual radiograph.

22. A method according to Claim 21, including the pre-hybridisation step for an initial period of time (during which the receptacle with a pre-hybridisation solution is rotated in the

chamber under the controlled temperature conditions), adding the radioactive probe solution to the receptacle which is then resealed and replaced in the chamber where it is rotated for a further period of time.

23. A method of detecting or investigating proteins, substantially as hereinbefore described with reference to the accompanying drawings.

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